Using Chimera to make Homology and Overlay Models Due Friday March 25

* Save the sequence of LDH Chain A from the 1LDN.pdb file. The easiest way to do this is to view the sequence at the RCSB.org site (“view sequence” in the top right corner of the 1LDN site) – but any other way that you find to do this is fine. I typically create a new Text Document, paste the sequence (in FASTA format) and save it with the .fasta tag instead of .txt.
* Do a Blast search (protein blast) to find homologues of *B. stearothermophilus* LDH in *Escherechia coli*. You’ll need to make sure you specify the appropriate organism.
* For your top hit, what is the % Identity and % similarity (positives)? Remind me, what do these values mean?
* On the ClustalW2 site, input the Bacillus and E. coli sequences and create a sequence alignment. You did this in your Bioinformatics exercise earlier in the term.
  + On the step 3 box, click on *more options* and select Pearson/FASTA as the output format.
  + Submit this project and wait for the alignment to complete. This should be very quick.
* Download the alignment file and paste it into a word document. Save this document in fasta format (this is really important – Chimera won’t recognize the .doc or .docx format as an alignment file). I typically create a new Text Document, paste the sequence (in FASTA format) and save it with the .fasta tag instead of .txt. Another option is to paste it in a Word document and save it as a .txt.
* Open this file in a new Chimera Session (make sure to select aligned FASTA as the format if prompted).
* Select Structure 🡪 Load Structures. This will load the 1LDN structure as long as you FASTA header (the first part that has the “>” contains the pdbID – which it will if you got the sequence from the rcsb site as suggested above. Go ahead and hide all chains except Chain A and then set the focus to Chain A.
* Go back to your alignment window. Select Structure 🡪 Modeller (homology).
* The target will be the *E. coli* protein and the template will be 1LDN\_A. Make sure these are selected.
* Now select ok. If you are asked to input a code for Modeller, use MODELIRANJE
* Wait for the process to complete – the progress is shown in the bottom left corner of the main Chimera window. Once complete, you should see several structure laid on top of the Bacillus LDH A chain. Picture These are all possible structures based on a complex algorithm that uses the alignment file you prepared.
  + What is the main difference between the modelled structures and the actual LDH structure? Are there regions that seem to align better then others? Why do you think this may be?
  + There will also be a new window that lists some information about each structure. Go ahead and select all but one and click on ‘hide’. This will leave you with a single structure mapped on 1LDN\_A. Picture.
* Refer back to questions 6 and 8 above. Using your alignment (seriously, it’s easier in the alignment file instead of looking at the homology model), determine if the *E. coli* enzyme has all the necessary residues to bind to NAD, Oxamic Acid, and F6P. Based on this, do you think that the *E. coli* enzyme can bind to each of the substrates? Clearly justify your answer.
* Zoom in on the active site (focus on the substrates) and show an image that confirms your answer for the previous question. Picture
* Zoom in on the allosteric site (focus on the substrates) and show an image that confirms your answer for the previous question. Picture
* Based on this exercise, do you think that FBP is an allosteric modifier of *E. coli* LDH like it is for the *Bacillus* enzyme?
* Redo this exercise for *Staphylococcus aureus.* Determine if FBP can be an allosteric modifier of LDH activity. Make sure to show a picture or two to clearly justify your hypothesis.

Your project:

Lethal factor (LF) is a protein (relative molecular mass 90,000) that is critical in the pathogenesis of anthrax. It is a highly specific protease that cleaves members of the mitogen-activated protein kinase kinase (MAPKK) family near to their amino termini, leading to the inhibition of one or more signaling pathways.

* Two structures of LF, together, are able to help biochemists figure out how this enzyme works. In the same chimera window, load 1JKY and 1J7N.
* Explore the structure of each of these. You’ll notice that one has the substrate peptide bound while the other does not. What does the other structure have in the substrate groove? Based on what you’ve learned about enzyme mechanisms, why might this be important?
* Our goal is to now get these two structure “matched” so that they can be directly compared. Using the Modeller will not be useful because either the substrate peptide or the other molecule will be omitted from the structure.
  + Open up the MatchMaker tool in the Structural Comparison menu (under tools).
  + Select a reference structure and the structure to match. If you do this wrong, you can always redo it.
* Now zoom in on the active site. Color the substrate one color and highlight the other molecule so that it is clearly visible.
* Select and show the sidechains of all amino acids within 5 angstroms of the substrate (an easy way to do this is to select the substrate and then use the “zone” tool under select – make sure to click the up arrow after doing the zone selection so that the whole amino acid is selected, not just the atom).
* Using these overlaid structures:
  + Predict the scissile bond (this is the peptide bond that gets hydrolyzed)
  + Predict the mechanism of the anthrax lethal factor. Make sure to consider the role of other amino acids that are in the active site.